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Previews

Quality Control of Receptor-Kinase Signaling Complexes

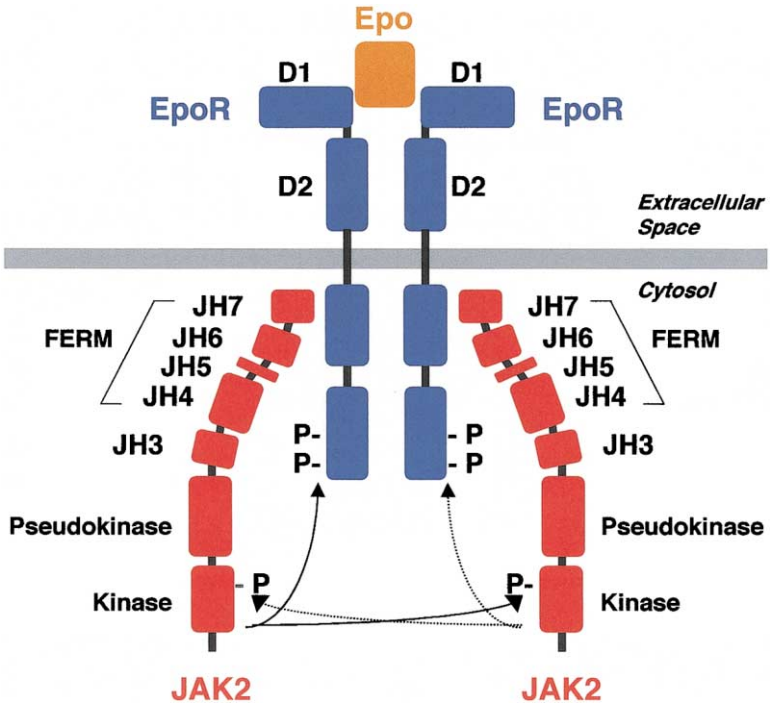
The erythropoietin receptor transduces signals leading to the growth, differentiation, and survival of red blood cell precursors via interaction with Janus kinase 2 (JAK2). This interaction was thought to occur only at the plasma membrane. Recent evidence, however, shows that JAK2 assembles with newly synthesized erythropoietin receptors in the endoplasmic reticulum, and that this assembly is essential for efficient expression of the receptors at the cell surface.

The binding of many extracellular ligands such as hormones, growth factors, cytokines, and antigens to their corresponding receptors on the cell surface triggers activation of intracellular tyrosine kinases. Some receptors (e.g., the receptors for insulin, epidermal growth factor, platelet-derived growth factor, vascular endothelial growth factor, and nerve growth factor) have intrinsic tyrosine kinase activity within their cytoplasmic domains. Other receptors (e.g., the T and B cell antigen receptors, immunoglobulin Fc receptors, and cytokine receptors) do not possess intrinsic tyrosine kinase activity but instead interact noncovalently with nonreceptor tyrosine kinases (e.g., Src family kinases and Janus family kinases) encoded by separate genes. These receptors are thought to travel unaccompanied from their site of synthesis in the endoplasmic reticulum (ER), through the secretory pathway and to the plasma membrane, where they encounter their partner tyrosine kinases, which have been synthesized in the cytoplasm. In this scenario, the kinases are not required for the biosynthetic transport of the receptors to the plasma membrane, and do not form obligatory stoichiometric complexes with the receptors. Although this model of assembly and transport may apply to some receptors, a study by Huang et al. published in the December issue of *Molecular Cell* reveals a startling new possibility. This report shows that the nonreceptor tyrosine kinase, Janus kinase 2 (JAK2), binds to the newly synthesized erythropoietin receptor (EpoR) in the ER and promotes its transport to the plasma membrane.

The EpoR is a member of the cytokine receptor family and plays a critical role in the development of red blood cells (Constantinescu et al., 1999). Binding of a single erythropoietin (Epo) molecule causes either association of two EpoR monomers or a conformational change of a preexisting EpoR homodimer (Wilson and Jolliffe, 1999; see Figure). This in turn triggers activation of the EpoR-associated JAK2, which phosphorylates itself as well as the EpoR. An intriguing property of the EpoR is the inefficiency with which the newly synthesized receptors are expressed at the cell surface, not only upon transfection into nonhematopoietic cells but also in normal erythropoietic precursors (Huang et al., 2001 and references therein). This inefficiency led Huang and colleagues to hypothesize that the availability of an unknown factor could limit the expression levels of EpoR at the cell surface. An obvious candidate for this factor was JAK2. In a series of elegant experiments, Huang et al. demonstrated that EpoR assembles with JAK2 while the receptor is still in the ER and that this assembly enhances receptor expression at the cell surface. The authors also mapped the interacting regions to a membrane-proximal segment of the EpoR cytoplasmic domain and the amino-terminal JH7 (JAK homology 7) domain of JAK2 (see Figure). A JAK2 mutant devoid of kinase activity was still able to enhance EpoR expression at the cell surface, indicating that the effects of JAK2 were due to its ability to assemble with the EpoR and not to its enzymatic activity.

Thus, in addition to serving as a signal transduction molecule, JAK2 behaves as a true subunit of the EpoR, essential for optimal surface expression of the receptor-kinase complex. This is likely a manifestation of mechanisms of quality control in the ER (Ellgaard et al., 1999). These mechanisms ensure that misfolded proteins or unassembled subunits of multiprotein complexes are retained in the ER and are, in many cases, retrotranslocated into the cytosol for degradation by the ubiquitin-proteasome pathway (Bonifacino and Weissman, 1998). It remains to be determined how EpoR lacking associated JAK2 is retained in the ER. One possibility is that the absence of JAK2 exposes a discrete determinant within the cytoplasmic domain of EpoR that is recognized as an ER retention signal by a specific recognition molecule. This would be analogous to the retention of the unassembled α subunit of the receptor for immunoglobulin E, which has a dilysine ER retrieval signal at the end of its cytoplasmic domain. Assembly with the γ subunit of this receptor masks the signal and releases the receptor from the ER (Letourneur et al., 1995). The EpoR, however, lacks a dilysine signal, and extensive mutagenesis of its JAK2 binding segment failed to reveal any other ER retention signal (Huang et al., 2001). An alternative possibility favored by the authors is that the absence of JAK2 causes the EpoR cytoplasmic domain to misfold, leading to recognition of this more global conformational change by the ER quality control machinery. In this model, JAK2 would assist in the folding of the EpoR cytoplasmic domain. This concept should open up a new avenue of inquiry into the mechanisms that sense protein misfolding on the cytoplasmic face of the ER membrane.

The EpoR-JAK2 complex may represent a paradigm for the biogenetic liaisons of at least a subset of receptors with their tyrosine kinase partners. Huang et al. provide a glimpse at the generality of this phenomenon by demonstrating that JAK2 also enhances the surface expression of a chimeric protein comprising the extracellular domain of the EpoR and the transmembrane and cytoplasmic domains of the prolactin receptor, another member of the cytokine receptor family functionally coupled to JAK2 (Huang et al., 2001). It will now be of interest



Schematic Representation of the Epo-EpoR-JAK2 Complex

EpoR assembles with JAK2 in the ER, and this assembly is required for optimal expression of the EpoR on the cell surface. Assembly involves interaction of a membrane-proximal segment of the EpoR cytoplasmic domain with the JH7 domain of JAK2. Binding of Epo to the EpoR-JAK2 complex results in JAK2 auto- or transphosphorylation as well as phosphorylation of the EpoR kinase domain (P). The D1 and D2 domains of the EpoR and JH3–JH7, pseudokinase and kinase domains of JAK2 are indicated. Residues 32–382 of JAK2 comprise a predicted FERM domain similar to those found in the cytoskeletal adaptor proteins moesin and radixin.

to investigate whether other types of receptors that activate nonreceptor tyrosine kinases are subject to similar regulatory controls. Even more tantalizing is the possibility that the requisite assembly of receptors with their cognate tyrosine kinases in the ER is not just a means of ensuring destruction of unproductive receptors generated under the artificial conditions of transfection, but also a mechanism for the posttranslational regulation of receptor expression during development.

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Selected Reading

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Regulating Sister Chromatid Separation by Separase Phosphorylation

A recent paper in *Cell* highlights a second mechanism of regulation of separase in addition to the bound inhibitor securin. This second pathway involves separase phosphorylation and is dependent on CDC2.

Faithful transmission of chromosomes from mother to daughter cells is fundamental for genetic inheritance. Separation of paired sister chromatids occurs during anaphase and needs to be properly coordinated to other cell cycle events. If sister chromatid separation and other mitotic events are not appropriately coupled, uncoordinated mitosis can take place, leading to errors in chromosome transmission and aneuploidy. Recent studies have made significant progress in understanding the molecular mechanism of sister chromatid separation (Uhlmann et al., 1999; Nasmyth et al., 2000; Yanagida, 2000).

Sister chromatids duplicated during S phase are closely connected to each other until anaphase. Sister chromatid cohesion is set up during S phase, and the sister chromatids are linked by a protein complex named cohesin.

In vertebrates, dissociation of cohesin from chromosomes is a two-step process. The majority of the cohesin detaches during prophase, and the remainder is removed in anaphase by cleavage of the cohesin subunit SCC1/Rad21 by a cysteine protease called separase.